Chloramination of nitrogenous contaminants (pharmaceuticals and pesticides): NDMA and halogenated DBPs formation

Julien Le Roux, Hervé Gallard, Jean-Philippe Croué

Chloramination of nitrogenous contaminants (pharmaceuticals and pesticides): NDMA and halogenated DBPs formation

JULIEN LE ROUX, HERVÉ GALLARD* AND JEAN-PHILIPPE CROUÉ[1]

Laboratoire de Chimie et Microbiologie de l'Eau (CNRS UMR 6008), Université de Poitiers – ENSIP, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France

* Corresponding author. Tel.: 33 (0)5 49 45 44 31; Fax: 33 (0)5 49 45 37 68

[1] Present address: King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia

E-mail addresses: julien.leroux@univ-poitiers.fr (J. Le Roux), herve.gallard@univ-poitiers.fr (H. Gallard), jp.croue@kaust.edu.sa (J.P. Croué)

Abstract

Disinfection with chloramines is often used to reduce the production of regulated disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs). However, chloramination can lead to the formation of N-nitrosamines, including N-nitrosodimethylamine (NDMA), a probable human carcinogen. Previous research used dimethylamine (DMA) as a model precursor of NDMA, but certain widely used tertiary dimethylamines (e.g. the pharmaceutical ranitidine) show much higher conversion rates to NDMA than DMA. This study investigates the NDMA formation potential of several tertiary amines including pharmaceuticals and herbicides. The reactivity of these molecules with monochloramine (NH_2Cl) is studied through the formation of NDMA, and other halogenated DBPs such as haloacetonitriles (HANs) and AOX (Adsorbable Organic Halides). Several compounds investigated formed NDMA in greater amounts than DMA, revealing the

importance of structural characteristics of tertiary amines for NDMA formation. Among these compounds, the pharmaceutical ranitidine showed the highest molar conversion to NDMA. The pH and dissolved oxygen content of the solution were found to play a major role for the formation of NDMA from ranitidine. NDMA was formed in higher amounts at pH around pH 8 and a lower concentration of dissolved oxygen dramatically decreased NDMA yields. These findings seem to indicate that dichloramine ($NHCl_2$) is not the major oxidant involved in the formation of NDMA from ranitidine, results in contradiction with the reaction mechanisms proposed in the literature. Dissolved oxygen was also found to influence the formation of other oxygen-containing DBPs (i.e. trichloronitromethane and haloketones). The results of this study identify several anthropogenic precursors of NDMA, indicating that chloramination of waters impacted by these tertiary amines could lead to the formation of significant amounts of NDMA and other non-regulated DBPs of potential health concern (e.g. dichloroacetonitrile or trichloronitromethane). This could be of particular importance for the chloramination of wastewater effluents, especially during water reuse processes.

Keywords

NDMA, Nitrosamine, Chloramination, Disinfection By-products, Ranitidine

1. Introduction

A large diversity of disinfection by-products (DBPs) are formed during water treatment processes using chlorination, including trihalomethanes (THMs) and haloacetic acids (HAAs). Disinfection with chloramines is known to significantly reduce the formation of regulated DBPs as compared to chlorination. However, chloramination favors the formation of *N*-nitrosamines, including *N*-nitrosodimethylamine (NDMA). The US Environmental Protection Agency classifies NDMA as a probable human carcinogen, evaluating a 10^{-6} risk level of cancer from NDMA concentration at 0.7 ng/L in drinking water (U.S. Environmental Protection Agency, 1987). Over the last decade, interest has been growing about NDMA formation during water treatment process. Several studies examined the mechanisms explaining the formation of NDMA during chlorination and chloramination. In most studies, dimethylamine (DMA) served as the model NDMA precursor (Choi and Valentine, 2002a; Choi and Valentine, 2002b; Choi and Valentine, 2003; Choi et al., 2002; Mitch and Sedlak, 2002; Schreiber and Mitch, 2005; Schreiber and Mitch, 2006). However, some studies indicated that the amount of dimethylamine present in surface waters (Gerecke and Sedlak, 2003) or secondary municipal wastewaters (Mitch and Sedlak, 2004) are not sufficient to explain the amount of NDMA formed. The role of tertiary amines presenting dimethylamine functional groups has been pointed out (Mitch and Sedlak, 2004; Schmidt et al., 2006). Recent studies looked at diuron as a precursor of NDMA. Results showed that the molar conversion rate is relatively low (< 1.5% of diuron forms NDMA) (Chen and Young, 2008; Chen and Young, 2009). Another tertiary amine ranitidine, a histamine antagonist widely used for peptic ulcer treatment was found to be an important NDMA precursor (62.9% NDMA yield obtained by Schmidt et al., 2006 and 89.9% by Shen and Andrews, 2011). Other tertiary amines led to less or equal NDMA formation than DMA, revealing the importance of structural characteristics of tertiary amine compounds for NDMA formation (Schmidt et al., 2006). Shen and Andrews (2011) demonstrated that several tertiary amines including pharmaceuticals and personal care products are

nitrosamine precursors during chloramines disinfection. According to these authors, the presence of electron donating group such as furan can increase the electron density on the nitrogen atom and then favors the reaction with chlorine leading to high NDMA yields observed with some pharmaceuticals (especially ranitidine). Ranitidine is sold worldwide as a gastrointestinal drug and has been detected at concentrations ranging from 70 ng/L to 540 ng/L in primary effluents of wastewater treatment plants (WWTP) in Spain (Radjenovic et al., 2009) and at ~10 ng/L in several surface waters (Kolpin et al., 2002; Zuccato et al., 2000). Ranitidine and other pharmaceuticals are not well removed by biological treatments and can be found in river waters receiving the WWTP effluents (Castiglioni et al., 2006; Radjenovic et al., 2009). Chloramination of wastewaters (e.g. for wastewater reuse purposes) impacted by pharmaceuticals is of great concern because of the potential risk of NDMA formation.

NDMA formation occurring during chloramination has previously been explained as a nucleophilic substitution reaction between monochloramine (NH_2Cl) and dimethylamine (DMA) to form an unsymmetrical dimethylhydrazine intermediate (UDMH) (Choi and Valentine, 2002b; Choi et al., 2002; Mitch and Sedlak, 2002). UDMH is then rapidly oxidized by NH_2Cl to NDMA at <3% yields. Over the past few years, studies have addressed the importance of chloramines speciation and dissolved oxygen (Schreiber and Mitch, 2006). Dichloramine ($NHCl_2$) was found to contribute to the production of NDMA during chloramine disinfection, occurring through the formation of a chlorinated UDMH (UDMH-Cl) as an intermediate rather than UDMH. Enhancing the formation of $NHCl_2$ by increasing the Cl:N ratio also lead to higher yields of NDMA during chloramination of tertiary amines (Shen and Andrews, 2011). Dissolved oxygen was also described as a critical parameter (Schreiber and Mitch, 2006). The authors proposed that the last step of the formation of NDMA consists in the incorporation of dissolved O_2 into UDMH-Cl, which would then lead to NDMA.

Degradation of tertiary amines may form other nitrogenous disinfection byproducts (N-DBPs) of potentially health concern, such as haloacetonitriles (HANs), halonitromethanes (HNMs), haloketones

97 (HKs) or cyanogen chloride (CNCl). HANs have been proved to be more toxic than HAAs and other
98 regulated DBPs (Muellner et al., 2007; Muellner et al., 2007). Trichloronitromethane (TCNM), also
99 known as chloropicrin, was the first of the HNMs to be identified as a DBP in drinking water (Hoigne
100 and Bader, 1988; Thibaud et al., 1987). Potential health effects of HNMs have already been studied
101 (National Cancer Institute, 1978; Schneider et al., 1999). They were found to be more mutagenic than
102 the corresponding halomethanes, and TCNM has been demonstrated to be particularly genotoxic (Plewa
103 et al., 2004). TCNM formation mechanisms have been proposed by chlorination and chloramination of
104 monomethylamine and n-propylamine (Joo and Mitch, 2007). TCNM formation is expected to increase
105 with pH during chlorination, and to be more important during chlorination than during chloramination.
106 TCNM formation from chlorination of lake waters was 40 times lower than that of chloroform (Hoigne
107 and Bader, 1988). Major haloketones (HKs) identified in chlorinated or chloraminated waters are
108 1,1-dichloro-2-propanone (1,1-DCP) and 1,1,1-trichloro-2-propanone (1,1,1-TCP). DCAN, 1,1-DCP
109 and CNCl formation were found to decrease when increasing pH, with maximum yields around pH 5-6
110 (Yang et al., 2007). DCAN formation during chloramination was much lower than during chlorination,
111 whereas CNCl and 1,1-DCP yields were higher in chloraminated water (Yang et al., 2007).
112 The goal of this study was to investigate the reactivity of several nitrogen-containing organic
113 compounds with monochloramine, through the formation of NDMA, HANs and AOX (Adsorbable
114 Organic Halides). Model compounds investigated included three herbicides (diuron, isoproturon,
115 trifluralin) and five pharmaceuticals: ranitidine (peptic ulcer treatment); doxepin and amitriptyline
116 (tricyclic antidepressants); mifepristone (an abortifacient) and minocycline (an antibiotic used for acne
117 treatment). All of them are tertiary amines presenting DMA functional groups. These anthropogenic
118 compounds are likely to enter natural waters via wastewater discharges (i.e. pharmaceuticals) or
119 agricultural runoff (i.e. herbicides). Because our objective was to study byproducts formation
120 mechanisms, solutions of model compounds were prepared at concentrations that are significantly

121 higher than what can be found in natural waters or wastewater effluents. As a result, DBPs were formed
122 at relatively high concentrations that are not likely to be found in treated waters. The influence of
123 several parameters (i.e. nitrites concentration, pH, chloramines speciation and dissolved oxygen
124 concentration) was investigated in order to better understand the reaction mechanisms that lead to the
125 formation of NDMA and some other DBPs (HANs, HKs, TCNM, AOX) during chloramination of
126 tertiary amines.
127
128 **2. Materials and methods**
129 **2.1. Materials**
130 All experiments were conducted using deionized water (Milli-Q, Millipore) buffered with sodium
131 acetate (pH = 4.0 - 5.5), a mixture of sodium phosphate monobasic and sodium phosphate dibasic
132 (pH = 7.0 - 8.5), or sodium carbonate (pH = 10). pH values were adjusted as needed using sodium
133 hydroxide or sulfuric acid (0.1 N, Fisher Scientific). Fluka Analytical methyl *tert*-butyl ether (>99%),
134 Fisher Scientific methylene chloride (GLC grade) and Carlo Erba methanol (>99.9%) were used without
135 further purification. Amitriptyline (>98%), diuron (>98%), doxepin (>98%), isoproturon (99.8%),
136 mifepristone (>98%), minocycline (92%, 8% water), ranitidine and trifluralin (>99%) were used without
137 further purification and were supplied through Sigma-Aldrich. Sodium hypochlorite (NaOCl, 13%,
138 Acros Organics) and ammonium chloride (Fisher Scientific, 99.9%) were used to prepare chloramine
139 reagents. Anhydrous sodium sulfite (Fisher Scientific) was used to quench residual chloramines.
140 Isotopically labeled standards, [6-^{2}H] *N*-nitrosodimethylamine (NDMA-d6, 98%, 1 mg.mL^{-1} in
141 methylene chloride) and [14-^{2}H] *N*-nitrosodi-n-propylamine (DPNA-d14, 98%, 1 mg.mL^{-1} in methylene
142 chloride) were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). A standard
143 solution containing seven *N*-nitrosamines (2000 µg/mL each in methylene chloride) was purchased from
144 Supelco (Sigma-Aldrich). The SPE materials used to extract nitrosamines from aqueous solutions

consisted in Supelclean™ prepacked coconut charcoal EPA 521 tubes, 2g/6ml, supplied from Supelco. A mixed standard containing haloacetonitriles (HANs), trichloronitromethane (TCNM) and haloketones (HKs) (EPA 551B Halogenated Volatiles Mix) and internal standard 1,2-dibromopropane were supplied from Supelco. All reagents not specified were obtained from Fisher Scientific.

2.2. Preparation and analysis of chloramines

Monochloramine (NH_2Cl) stock solutions were prepared daily by slowly adding sodium hypochlorite (NaOCl) into a rapidly stirred ammonium chloride (NH_4Cl) solution adjusted to pH = 8.5 with sodium hydroxide, and using a Cl:N molar ratio of at least 1:1.2 to avoid breakpoint chlorination resulting from local excess of hypochlorite (Mitch and Sedlak, 2002). Adjusting the pH at 8.5 minimizes the disproportionation of NH_2Cl to dichloramine ($NHCl_2$), since $NHCl_2$ forms at pH < 8 (U.S. Environmental Protection Agency, 1999) according to the equilibrium:

$$2NH_2Cl + H^+ = NHCl_2 + NH_4^+ \quad (1)$$

Free chlorine and total chlorine concentrations in the stock solutions of sodium hypochlorite were determined iodometrically with sodium thiosulfate 0.1 M (Prolabo, >99.9%). Initial NH_2Cl and $NHCl_2$ concentrations were determined by spectrophotometric measurement using their respective molar extinction coefficients at 245 nm and 295 nm and solving simultaneous equations (Schreiber and Mitch, 2005). Residual chloramines were analyzed iodometrically (Eaton et al., 1995).

2.3. Chloramination experiments

All glassware used during these experiments was washed with deionized water and baked at 500 °C for at least 5 hours prior to use. Reactions were conducted in sealed 1 L amber glass bottles at 20 °C in a temperature-controlled room, under dark conditions to avoid photolysis of NDMA. Chloramination experiments were conducted following the approach of Mitch et al. (Mitch et al., 2003), using high

concentrations of NH_2Cl (200 to 300 mg/L as Cl_2) and a reaction time of 5 days for most of our experiments. NH_2Cl remained in excess during all the reaction time. Solutions were prepared by dissolving a pre-determined amount of compound in 1 L of 10 mM acetate, phosphate or carbonate buffer. 100 mL of preformed monochloramine was then added to the working solution. Chloramination experiments were conducted in triplicate. All series of experiments were completed with the chloramination of a corresponding blank solution.

At given contact times, 350 mL of samples were transferred for residual chlorine, HANs and AOX analyses, and 750 mL were processed for nitrosamines analyses.

Percent molar yields were calculated using the initial molar concentration of the studied compounds, following Equation 2.

$$\text{DBP yield (\%)} = \frac{[\text{DBP}]\,(\text{nM})}{[\text{Organic compound}]_0\,(\text{nM})} \times 100 \quad (2)$$

AOX formation rates were calculated as follows:

$$\text{AOX formation rate (mol/mol)} = \frac{[\text{AOX}]\,(\mu\text{g/L as Cl}) / 35.5}{[\text{Organic compound}]_0\,(\mu\text{M})} \quad (3)$$

2.4. Influence of dissolved O_2

Experiments were performed in saturated dissolved O_2 solution and in absence of oxygen. The removal of oxygen was operated prior to chloramination by bubbling nitrogen gas through a Teflon line until dissolved O_2 concentration was less than 0.3 mg O_2/L. The dissolved O_2 concentration was monitored using a WTW Oxi 330 oxygen meter. The samples were continuously bubbled until the end of the experiment (2 h contact time). Previous experiments were conducted with NDMA standard solutions in

order to verify that nitrogen bubbling for 2 hours did not lead to any significant NDMA or chlorinated DBPs stripping.

2.5. Analytical methods

2.5.1. Nitrosamines analysis

NDMA analysis was performed according to the US EPA method (U.S. Environmental Protection Agency, 2004), consisting in a solid-phase extraction (SPE) using coconut charcoal EPA 521 tubes followed by GC/MS analysis in EI mode. Analytical details are provided elsewhere (Le Roux et al., 2010) and summarized below. Chloramination reactions were quenched using 2.5 g sodium sulfite before SPE. Unlike previous studies (Chen and Young, 2009; Schreiber and Mitch, 2006), no ascorbic acid was used because it was found to degrade into furfural during SPE in coconut charcoal tubes, which led to poor NDMA recovery. Prior to the extraction, 200 ng of NDMA-d6 was added to each 1 L sample as an internal standard. Each sample was extracted at a continuous flow rate through the SPE tube. Analytes were eluted from the SPE bed with 15 mL of methylene chloride. Extracts were then filtered through 5 g anhydrous sodium sulfate column to remove residual water. Methylene chloride extracts were then concentrated down to 1 mL under a stream of N_2, after addition of DPNA-d14 (200 ng) used as recovery standard. Samples extracts were analyzed immediately after SPE using a HP 6890 series gas chromatograph system coupled with a HP 5973 mass selective detector (MSD) in electron impact (EI) mode. Samples were injected in pulsed splitless mode using helium as the carrier gas. A Varian VF-5ms capillary column (30 m by 0.25 mm i.d. by 0.25 µm film thickness) supplied through Interchim was used to separate the analytes. Quantitative analyses were performed in selected-ion monitoring (SIM) mode. Full scan mode (40 - 240 m/z) analyses were also conducted for complementary spectral information. This method reached extraction efficiencies of approximately

213 85%. The method detection limit (MDL) for NDMA at the 99% confidence level was determined to be
214 33 ng/L.

215 **2.5.2. HANs, HKs, TCNM and AOX analysis**

216 Chloramination reactions were stopped using 250 mg ascorbic acid prior to HAN and AOX analysis to
217 avoid HANs degradation occurring in the presence of excess sodium sulfite (Croue and Reckhow,
218 1989). HANs, HKs and TCNM analysis was based on the US EPA 551.1 method (Munch and Hautman,
219 1995). 50 mL of samples were transferred to amber glass bottles and 1,2-dibromopropane (100 µg/L)
220 was added as an internal standard. Samples were extracted by shaking for 4 min into 3 mL MTBE.
221 Extracts were analyzed using GC/MS (same equipment as used for nitrosamines analysis), along with
222 HANs, HKs and TCNM calibration standards. 1 µL was injected in pulsed splitless mode with an inlet
223 temperature of 150 °C. The column temperature program was 40 °C held for 3 min, ramping to 55 °C at
224 2 °C/min and holding for 1 min, then a ramp of 5 °C/min to 85 °C, and a final ramp of 40 °C/min to
225 200 °C held for 1 min. The MDL for this method is about 0.1 µg/L. AOX were determined using a
226 Dohrmann DX 20 analyzer after adsorption onto activated carbon (European Standard EN 1485, 1996).
227 The detection limit for this method is about 20 µg as Cl^-/L.
228

229 **3. Results and discussion**

230 Figure 1 shows as example the kinetic results for AOX, DCAN and NDMA formation obtained with
231 3 µM ranitidine and 2.5 mM NH_2Cl at pH 8.5. NH_2Cl consumption over 120 hours was always about
232 50% of the initial concentration. Same results were obtained for the other investigated compounds.
233 Results from control samples exhibited similar chloramine decay. Kinetic modeling performed using
234 Copasi software and Jafvert and Valentine's model (Jafvert and Valentine, 1992) confirmed that
235 monochloramine (NH_2Cl) predominantly decays by self-disproportionation under our experimental
236 conditions (pH 8.5, 10 mM phosphate buffer). Hence, the consumption of NH_2Cl by the model

237 compounds investigated was insignificant and could not be quantified. AOX formation leveled off after
238 only 2 h contact time, whereas NDMA and DCAN formation were slower and reached their maximum
239 after 24 h. This observation is in accordance with results from the chlorination of proteins (one of the
240 most important precursors of DCAN in drinking waters), that shows a two-step process (Reckhow,
241 2001). First, rapid reactions with reactive sites form THMs and Total Organic Halides (TOX) (Hureiki
242 et al., 1994), then slow degradation of proteins leads to DCAN formation. A similar behavior for DBPs
243 formation kinetics could occur during the chloramination of ranitidine.
244
245 The formation of NDMA, HANs and AOX at pH 8.5 from selected compounds was monitored after 5
246 days of contact time (Table 1). Ranitidine exhibited the highest molar yield with 40.2% NDMA formed.
247 Similar amounts of NDMA were produced after 5 days of contact time for initial monochloramine
248 concentrations of 0.5 mM to 2.5 mM and 100 nM ranitidine solutions i.e. for large excess of
249 monochloramine. Yields for the other pharmaceuticals ranged from 0.4 to 8.2% and less than 0.4% for
250 diuron and isoproturon. NDMA formation from DMA is known to be < 3% molar conversion (Schmidt
251 et al., 2006; Schreiber and Mitch, 2006) Minocycline and especially ranitidine exhibited higher molar
252 yields than other tertiary amines or DMA.
253 Compounds presenting heterocyclic ring in their structure (e.g. furan in ranitidine) produced more
254 NDMA than compounds with DMA functions near carbonyl groups (i.e. diuron and isoproturon)
255 (Schmidt et al., 2006) and compounds with aromatic rings (e.g. minocycline or mifepristone).
256 According to Shen and Andrews (2011), the higher yield observed for ranitidine would be explained by
257 the electron-donating effect of furan group that increases electron density on the nitrogen atom and thus
258 enhance electrophilic substitution of chlorine atom. This mechanism would involve the formation of
259 dimethylchloramine (DMCA), DMA and then NDMA (Mitch and Sedlak, 2004). However, the presence
260 of DMA as a key intermediate could not explain the high yield obtained with ranitidine because the

NDMA yields from DMA are always < 3% in literature. An alternative mechanism would involve the nucleophilic substitution of NH_2Cl on nitrogen atom instead of electrophilic substitution (i.e. chlorine transfer with formation of a DMCA group). Further research is needed to fully address the formation mechanism of NDMA from dimethylaminomethylfuran group.

As suggested by (Shen et Andrews, 2011), ranitidine can be considered as a significant NDMA precursor because 6 to 39% of ranitidine is excreted as the parent form by human body (Jjemba, 2006) and its metabolites maintain the furan and DMA groups in their structures. Moreover, the removal of ranitidine through WWTP can be relatively low (Castiglioni et al., 2006). The presence of ranitidine and its metabolites in wastewaters could contribute significantly to the high NDMA formation potentials observed at several WWTP, which are much higher than concentrations predicted based upon DMA concentrations in raw waters and calculated following previously proposed formation mechanisms (Mitch and Sedlak, 2004; Mitch et al., 2003).

Minocycline, the second highest NDMA precursor of the pool of compounds studied (8.2% NDMA molar conversion) contains two dimethylamine functional groups that probably partly explain the significant formation of NDMA. Amitriptyline and doxepin have similar molecular structures and formed 1.15 and 2.32% of NDMA, respectively. The three carbon atoms between the DMA group and the three rings could explain their lower reactivity compared to ranitidine (Shen and Andrews, 2011). The presence of the oxygen atom in doxepin would explain the higher yield of NDMA for this molecule compared to amitriptyline. Chloramination of trifluralin led to the formation of 0.18% DPNA, half the formation of NDMA obtained from mifepristone that also incorporates an aromatic ring substituted with a dialkylamine group. The lower yield for trifluralin can be attributed to the electron withdrawing effect of the two nitro groups. The electron withdrawing effect of the carbonyl group would also explain the low formation yield of NDMA from isoproturon and diuron (Schmidt et al., 2006).

284 In full scan mode, the GC/MS chromatogram of the extracts revealed the presence of
285 dimethylformamide (DMF) and dimethylcyanamide (DMC) as by-products of the reaction of
286 monochloramine with diuron or ranitidine. These compounds are known to be UDMH oxidation
287 products, as well as NDMA (Mitch and Sedlak, 2002). However, formation mechanisms of DMC and
288 DMF remain unclear. No other nitrosamine was detected during the experiments with compounds
289 containing dimethylamine functional groups.
290
291 Ranitidine formed about 10 times less DCAN than NDMA (Table 1). Minocycline was the second
292 highest DCAN precursor (1.5% DCAN yield). For the other compounds studied, the amounts of DCAN
293 formed were quite similar to those of NDMA (< 1% yield). No TCAN formation was detected during
294 these experiments. No correlation could be made between NDMA formation and DCAN formation but
295 more DCAN was generally formed when NDMA was produced in higher amounts. Minocycline
296 exhibited the highest AOX formation rate (8.98 mol/mol), which could be related to its highly aromatic
297 and oxygen-containing structure. Ranitidine was the second AOX precursor with 0.95 mol/mol
298 formation rate. The other compounds investigated did not lead to any significant AOX formation in our
299 experimental conditions. These results indicate that compounds producing high amount of NDMA tend
300 also to form more of other DBPs (AOX, and especially DCAN).
301
302 **3.1. Influence of Nitrites**
303 Previous research (Choi and Valentine, 2003) proposed an “enhanced nitrosation pathway” describing
304 NDMA formation from the reaction of DMA with nitrite and hypochlorite. Nitrites were also found to
305 enhance the formation of NDMA during the chlorination of diuron (Chen and Young, 2009). Because
306 low amount of free chlorine may be present in monochloramine solution, nitrites could contribute to the
307 formation of NDMA by chloramination. Experiments conducted with 1 µM amitriptyline and 1 µM

mifepristone showed that NDMA formation was not significantly different in presence and in absence of 1 µM nitrites (Table 2). These results indicate that the formation of NDMA from tertiary amines during chloramination is not enhanced by any nitrosation mechanism, which could have occurred in presence of free chlorine and nitrites.

3.2. Effect of pH

To assess the influence of pH on the formation of DBPs, NH_2Cl (2.5 mM) was applied to ranitidine solutions (3 µM) in deionized water buffered at pH ranging from 4 to 10 (Table 3). NDMA, HANs, HKs, and TCNM were analyzed after a contact time of 5 days. NDMA formation from chloramination of ranitidine exhibited a maximum (59.6% yield) at pH 7.9, which is similar to 62.9% reported in Schmidt et al. (2006) for the same conditions. Amitriptyline and mifepristone followed similar trends, forming much less NDMA at pH 10 than at pH 8 (Table 2). Other studies showed that NDMA formation from chloramination of DMA or diuron varied with pH with a maximum formation rate between pH 7 and 9 (Chen and Young, 2008; Mitch and Sedlak, 2002; Schreiber and Mitch, 2006). Self-decomposition and hydrolysis of NH_2Cl at pH < 8 are known to lead to the formation of $NHCl_2$ (Valentine and Jafvert, 1988). Because $NHCl_2$ is known to enhance NDMA formation (Schreiber and Mitch, 2006), then acid-catalyzed disproportionation of NH_2Cl into $NHCl_2$ could explain the higher formation of NDMA at pH 7.9 compared to pH > 8. However, kinetic modeling of NH_2Cl decomposition indicates that $NHCl_2$ is not present in important amounts at pH 7.9. Furthermore, NDMA formation from ranitidine at pH where $NHCl_2$ is the major specie (i.e. pH ~ 4) was much lower than at pH 8, indicating that other factors than chloramines speciation may play a role in NDMA formation mechanisms. Thus, ranitidine acid-base equilibrium (pKa = 8.2) could explain the decrease of NDMA formation when the protonated form of ranitidine decreases at pH > 8 (Figure 2). At pH < 8, NDMA

331 formation seems to be strongly dependent on the NH_2Cl concentration in the solution, and was not
332 enhanced by the presence of $NHCl_2$.
333 As shown in Table 3, important amounts of trichloronitromethane (TCNM) were formed from ranitidine
334 at acidic pH (12.57% at pH 4). The amounts of TCNM formed decreased as the pH was raised from
335 pH 4 to pH 10, but were still higher than other chlorinated DBPs at neutral and basic pH. Whereas
336 NDMA formation was maximum around pH 8, DCAN, 1,1-DCP and 1,1,1-TCP exhibited a maximum
337 formation yield at pH 7. Moreover, TCAN formation from ranitidine was low and relatively constant
338 when varying pH from 4 to 10. The lower concentrations of DCAN and 1,1-DCP at pH > 7 can be
339 explained by base-catalyzed decomposition (Croue and Reckhow, 1989; Reckhow, 2001; Yang et al.,
340 2007).
341 AOX formation was constant from pH 4 to 7 and then decreased at alkaline pH (Table 3). As shown in
342 Figure 3, analyzed DBPs represent only a few percent of the AOX formed. TCNM accounts for 20% of
343 the produced AOX at pH 4. However, the proportion of identified DBPs is decreasing when increasing
344 pH.
345

346 **3.3. Influence of dichloramine**

347 To evaluate the influence of $NHCl_2$ on NDMA formation from ranitidine, preformed $NHCl_2$ or NH_2Cl
348 (1 mM) were applied to ranitidine solutions. Previous research indicated that NDMA formation from
349 DMA and $NHCl_2$ was much higher than in the presence of NH_2Cl (Schreiber and Mitch, 2006). Our
350 results showed that NDMA formation from 100 nM ranitidine buffered at pH 8 and after 24 h was
351 significantly lower with $NHCl_2$ than with NH_2Cl (46.8% and 80.2% molar yields respectively,
352 Figure 4).
353 Total chlorine decay during our experiments with $NHCl_2$ was about 85% after 24 hours, while it was
354 only 25% with NH_2Cl. Thus, $NHCl_2$ decomposition is more rapid than NH_2Cl at pH around pH 8, which

could explain why less NDMA was formed in presence of $NHCl_2$. The autodecomposition of $NHCl_2$ in our experiments could be well simulated by the kinetic model of Jafvert and Valentine (1992). According to this model, the hydrolysis of dichloramine (Equation 4) and inverse dismutation (Equation 5) lead to the formation of significant amounts of monochloramine.

$$NHCl_2 + H_2O \rightarrow NH_2Cl + HOCl \quad (4)$$

$$NHCl_2 + NH_3 + H^+ \rightarrow 2\ NH_2Cl + H^+ \quad (5)$$

The use of the model showed that the residual chlorine concentrations of 0.3 mM analyzed after 24h of contact time could be explained by the formation of NH_2Cl from $NHCl_2$ decomposition, which is almost complete after 24h. In this condition, the simulated NH_2Cl exposure (i.e. the C.t value) represents about 38% of the NH_2Cl exposure from direct NH_2Cl addition. Thus, NH_2Cl formed from the decomposition of $NHCl_2$ could explain the amounts of NDMA formed during the chloramination of ranitidine using dichloramine. These results seem to indicate that dichloramine would not be involved into the formation of NDMA from ranitidine. No significant differences were observed for DCAN formation after the application of either NH_2Cl or $NHCl_2$ to 100 nM ranitidine at pH 8 (Figure 4).

3.4. Influence of dissolved oxygen

It has been demonstrated that dissolved oxygen concentration plays a major role in the formation of NDMA by chloramination of DMA (Schreiber and Mitch, 2006). Moreover, a recent study showed that the formation of NDMA from DMA could be catalyzed by activated carbon, and that the presence of oxygen was a critical factor in this mechanism (Padhye et al., 2010).

In order to assess whether or not dissolved oxygen would influence the formation of NDMA from the chloramination of other model compounds, 2.7 mM NH_2Cl was applied to 3 µM ranitidine during 2h in presence and in absence of dissolved O_2. NDMA formation was significantly inhibited for low oxygen

concentration (~ 0.2 mg O_2/L) compared to ambient O_2 concentration (~ 9 mg O_2/L) (molar yields of 4.01% and 54% respectively, Figure 5a).

Dissolved O_2 concentration did not affect AOX formation as much as NDMA formation (Figure 5b). Moreover, DCAN formation was not influenced by dissolved O_2 concentration while the formation of other halogenated DBPs containing oxygen atoms (nitro or ketones functional groups, i.e. TCNM, 1,1-DCP and 1,1,1-TCP) was approximately an order of magnitude lower in the presence of 0.2 mg O_2/L (Figure 6). This indicates that dissolved oxygen could be incorporated into those DBPs, i.e. an oxygen atom of dissolved oxygen could serve as a source for the oxygen atom in nitroso or ketone functions of DBPs. Further research with model compounds and using inhibitors of oxygen species need to be done to elucidate the mechanisms involved into the formation of NDMA and to understand the role of dissolved oxygen.

4. Conclusion

- Even if concentrations of compounds used for our study were relatively high and are not likely to be found in natural waters, we observed that several nitrogenous anthropic compounds can lead to important concentrations of N-DBPs including NDMA, DCAN, 1,1-DCP, or TCNM. From the seven compounds investigated in our study, four compounds contain dimethylamine functional groups and exhibited yields higher than 1.15% (ranitidine, minocycline, doxepin, amitriptyline). Especially, the pharmaceutical ranitidine is of great concern regarding its high molar yield into NDMA (~60% at pH 7.9), as shown in earlier studies.
- Such differences in NDMA formation can not be explained by the release of DMA and the reactions of DMA with chloramines. More simple compounds than those described in the present work need to be studied to improve our understanding of molecular structure influence on the formation of NDMA.

- Our results demonstrate that the reaction of $NHCl_2$ with ranitidine would not form more NDMA than NH_2Cl. However, we confirmed the implication of dissolved oxygen in NDMA formation mechanisms. Dissolved oxygen was found to play a role into the formation of other oxygen-containing DBPs (TCNM, 1,1-DCP and 1,1,1-TCP) but did not influence DCAN formation. These results need further investigation to better understand the incorporation of dissolved oxygen into DBPs.
- Considering the high conversion of ranitidine to NDMA, the use of chloramination as a disinfection for wastewaters containing ranitidine can lead to the formation of important amounts of NDMA. This could explain the high NDMA formation potentials observed at several WWTP, which are much higher than concentrations predicted based upon DMA concentrations in raw waters.

Acknowledgement

We would like to thank the French Ministry of Higher Education and Research (Ministère de l'Enseignement Supérieur et de la Recherche) for its financial support.

426

427 **References**

428 Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of
429 Pharmaceuticals in Sewage Treatment Plants in Italy. Environ. Sci. Technol. 40 (1), 357-363.

430 Chen, W., Young, T.M., 2008. NDMA formation during chlorination and chloramination of aqueous
431 diuron solutions. Environ. Sci. Technol. 42 (4), 1072-1077.

432 Chen, W., Young, T.M., 2009. Influence of nitrogen source on NDMA formation during chlorination of
433 diuron. Water Res. 43 (12), 3047-3056.

434 Choi, J., Duirk, S.E., Valentine, R.L., 2002. Mechanistic studies of N-nitrosodimethylamine-(NDMA)
435 formation in chlorinated drinking water. J. Environ. Monit. 4 (2), 249-252.

436 Choi, J., Valentine, R.L., 2002. A kinetic model of N-nitrosodimethylamine (NDMA) formation during
437 water chlorination/chloramination. Water Sci. Technol. 46 (3), 65-71.

438 Choi, J., Valentine, R.L., 2002. Formation of N-nitrosodimethylamine (NDMA) from reaction of
439 monochloramine: A new disinfection by-product. Water Res. 36 (4), 817-824.

440 Choi, J., Valentine, R.L., 2003. N-Nitrosodimethylamine Formation by Free-Chlorine-Enhanced
441 Nitrosation of Dimethylamine. Environ. Sci. Technol. 37 (21), 4871-4876.

442 Croue, J.P., Reckhow, D.A., 1989. Destruction of chlorination byproducts with sulfite. ESandT
443 Contents 23 (11), 1412-1419.

444 Eaton, A.D., Clesceri, L.S., Greenberg, A.E., Eds. *Standard Methods for the Examination of Water and*
445 *Wastewater*, 19th ed.; American Public Health Association/American Water Works Association/Water
446 Environment Federation Publishers; Washington D.C., USA, 1995.

447 European Standard EN 1485 (1996) Water Quality Determination of Adsorbable Organically Bound
448 Halogens (AOX).

449 Gerecke, A.C., Sedlak, D.L., 2003. Precursors of N-nitrosodimethylamine in natural waters. Environ.
450 Sci. Technol. 37 (7), 1331-1336.

451 Hoigne, J., Bader, H., 1988. The formation of trichloronitromethane (chloropicrin) and chloroform in a
452 combined ozonation/chlorination treatment of drinking water. Water Res. 22 (3), 313-319.

453 Hureiki, L., Croué, J., Legube, B., 1994. Chlorination studies of free and combined amino acids. Water
454 Res. 28 (12), 2521-2531.

455 Jafvert, C.T., Valentine, R.L., 1992. Reaction scheme for the chlorination of ammoniacal water.
456 Environ. Sci. Technol. 26 (3), 577-786.

457 Jjemba, P.K., 2006. Excretion and ecotoxicity of pharmaceutical and personal care products in the
458 environment. Ecotoxicol. Environ. Saf. 63 (1), 113-130.

459 Joo, S.H., Mitch, W.A., 2007. Nitrile, Aldehyde, and Halonitroalkane Formation during
460 Chlorination/Chloramination of Primary Amines. Environmental Science & Technology 41 (4), 1288-
461 1296.

462 Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T.,
463 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams,
464 1999−2000: A National Reconnaissance. Environmental Science & Technology 36 (6), 1202-1211.

465 Le Roux, J., Gallard, H., Croue, J.P., 2010. Formation of NDMA by Chloramination of Nitrogenous
466 Pharmaceuticals. Water Practice & Technology 5 (4).

467 Mitch, W.A., Gerecke, A.C., Sedlak, D.L., 2003. A N-Nitrosodimethylamine (NDMA) precursor
468 analysis for chlorination of water and wastewater. Water Res. 37 (15), 3733-3741.

469 Mitch, W.A., Sedlak, D.L., 2002. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine
470 during chlorination. Environ. Sci. Technol. 36 (4), 588-595.

471 Mitch, W.A., Sedlak, D.L., 2004. Characterization and Fate of N-Nitrosodimethylamine Precursors in
472 Municipal Wastewater Treatment Plants. Environ. Sci. Technol. 38 (5), 1445-1454.

473 Muellner, M.G., Wagner, E.D., Mccalla, K., Richardson, S.D., Woo, Y., Plewa, M.J., 2007.
474 Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic? Environ.
475 Sci. Technol. 41 (2), 645-651.

476 Munch, D.J., Hautman, D.P. (1995) EPA Method 551.1 Determination of chlorination disinfection
477 byproducts, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-
478 liquid extraction and gas chromatography with electron-capture detection, revision 1.0. Available online
479 from http://www.epa.gov/sam/pdfs/EPA-551.1.pdf, accessed April 22, 2009.

480 National Cancer Institute (1978) Bioassay of chloropicrin for possible carcinogenicity (CAS No. 76-06-
481 2). *National Toxicology Program Carcinogenesis Technical Report Series, U.S. Department of Health*
482 *and Human*.

483 Padhye, L., Wang, P., Karanfil, T., Huang, C., 2010. Unexpected role of activated carbon in promoting
484 transformation of secondary amines to N -nitrosamines. Environ. Sci. Technol. 44 (11), 4161-4168.

485 Plewa, M.J., Wagner, E.D., Jazwierska, P., Richardson, S.D., Chen, P.H., McKague, A.B., 2004.
486 Halonitromethane drinking water disinfection byproducts: Chemical characterization and mammalian
487 cell cytotoxicity and genotoxicity. Environ. Sci. Technol. 38 (1), 62-68.

488 Radjenovic, J., Petrovic, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater
489 and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor
490 (MBR) treatment. Water Res. 43 (3), 831-841.

491 Reckhow, D.A., MacNeill, A.L., Platt, T.L., MacNeill, A.L., McClellan, J.N., 2001. Formation and
492 degradation of dichloroacetonitrile in drinking waters. J. Water Supply Res. Technol. AQUA 50 (1), 1-
493 13.

494 Schmidt, C.K., Sacher, F., Brauch, H. (2006) Strategies for minimizing formation of NDMA and other
495 nitrosamines during disinfection of drinking water. Proceedings of the American Water Works
496 Association Water Quality Technology Conference, Denver, CO.

497 Schneider, M., Quistad, G.B., Casida, J.E., 1999. Glutathione activation of chloropicrin in the
498 Salmonella mutagenicity test. Mutation Research/Genetic Toxicology and Environmental Mutagenesis
499 439 (2), 233-238.

500 Schreiber, I.M., Mitch, W.A., 2005. Influence of the order of reagent addition on NDMA formation
501 during chloramination. Environ. Sci. Technol. 39 (10), 3811-3818.

502 Schreiber, I.M., Mitch, W.A., 2006. Nitrosamine formation pathway revisited: The importance of
503 chloramine speciation and dissolved oxygen. Environ. Sci. Technol. 40 (19), 6007-6014.

504 Shen, R., Andrews, S., 2011. Demonstration of 20 pharmaceuticals and personal care products (PPCPs)
505 as nitrosamine precursors during chloramine disinfection. Water Res. 45 (2), 944-952.

506 Thibaud, H., de Laat, J., Merlet, N., Doré, M., 1987. Chloropicrin formation in aqueous solution: Effect
507 of nitrites on precursors formation during the oxidation of organic compounds (Formation de
508 chloropicrine en milieu aqueux: Influence des nitrites sur la formation de precurseurs par oxydation de
509 composes organiques). Water Res. 21 (7), 813-821.

510 U.S. Environmental Protection Agency (1987) Integrated Risk Information System (IRIS), N-
511 nitrosodimethylamine. Office of Research and Development (ORD), National Center for Environmental
512 Assessment. Available online from www.epa.gov/iris/subst/0045.htm, accessed October 20, 2008.

513 U.S. Environmental Protection Agency (1999) Alternative disinfectants and oxidants. EPA guidance
514 manual. Available online from
515 http://www.epa.gov/OGWDW/mdbp/alternative_disinfectants_guidance.pdf, accessed April 10, 2009.

516 U.S. Environmental Protection Agency (2004) Method 521: determination of nitrosamines in drinking
517 water by solid phase extraction and capillary column gas chromatography with large volume injection
518 and chemical ionization tandem mass spectrometry (MS/MS). EPA/600/R-05/054. Available online
519 from http://www.epa.gov/nerlcwww/m_521.pdf, accessed October 20, 2008.

520 Valentine, R.L., Jafvert, C.T., 1988. General acid catalysis of monochloramine disproportionation.
521 Environmental Science & Technology 22 (6), 691-696.

522 Yang, X., Shang, C., Westerhoff, P., 2007. Factors affecting formation of haloacetonitriles, haloketones,
523 chloropicrin and cyanogen halides during chloramination. Water Res. 41 (6), 1193-1200.

524 Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the
525 environment. The Lancet 355 (9217), 1789-1790.

526

527

528

529

530

531

532

533

534

535

536

537

538

539

540

541

542

543

544

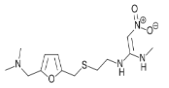
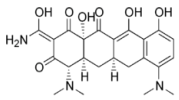
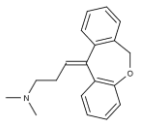
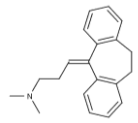
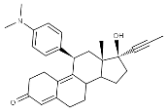
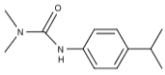
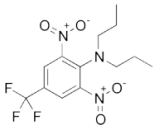
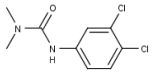
545

546

547 **Tables**

548 Table 1. Nitrosamine and DCAN formation from compounds investigated at pH 8.5 (5 days contact

549 time)

Compound investigated	Molecular structure	Compound concentration (nM)	NH_2Cl concentration[a] (mM)	Molar yield[b] (%) (SD[c])		AOX formation rate[e] (mol/mol) (SD[c])
				Nitrosamine[d]	DCAN	
Ranitidine		14760	4.0	40.2 (1.4)	5.8 (0.2)	0.95 (0.18)
Minocycline		2820	2.5	8.2 (0.7)	1.5 (0.1)	8.98 (0.89)
Doxepin		1730	2.5	2.32 (0.01)	0.5 (0.1)	N.D.
Amitriptyline		3480	2.5	1.15 (0.04)	0.8 (0.4)	N.D.
Mifepristone		3170	2.5	0.39 (0.02)	0.2 (0.1)	N.D.
Isoproturon		5290	2.5	0.34 (0.02)	N.D.	N.D.
Trifluralin		810	2.5	0.18 (0.01)	N.D.	N.D.
Diuron		16560	4.0	0.15 (0.01)	N.D.	N.D.

[a] Initial NH_2Cl concentration applied to a solution containing a compound investigated in deionized water with 10 mM phosphate buffer (pH 8.5)
[b] Molar yields were calculated based upon the initial compound concentration
[c] SD = Standard Deviation on 3 replicates
[d] Nitrosamine formed is NDMA except for trifluralin (DPNA)
[e] AOX formation rates expressed as mol AOX as Cl^- / mol of initial compound
N.D. = Not Detected

550 Table 2. Effect of pH and NO_2^- on NDMA formation from amitriptyline and mifepristone over 5 days

551 with 10 mM buffer (phosphate for pH 8.5 and carbonate for pH 10).

Expt	Compound investigated	Compound concentration (µM)	NH_2Cl concentration (mM)	NDMA yield[a] (%) (SD[b])	pH
1	Amitriptyline	0.38	3.8	2.37 (0.34)	8.5
		0.38	3.8	0.08 (0.01)	10
	Mifepristone	0.35	3.8	1.00 (0.30)	8.5
		0.35	3.8	0.04 (0.01)	10
2	Amitriptyline	1	3.4	1.93 (0.15)	8.5
	Amitriptyline + 1 µM NO_2^-	1	3.4	1.72 (0.15)	8.5
	Mifepristone	1	3.4	0.89 (0.09)	8.5
	Mifepristone + 1 µM NO_2^-	1	3.4	0.97 (0.09)	8.5

[a]Molar yields were calculated based upon the initial compound concentration
[b]SD = Standard Deviation on 3 replicates

552

553

554

555

556

557

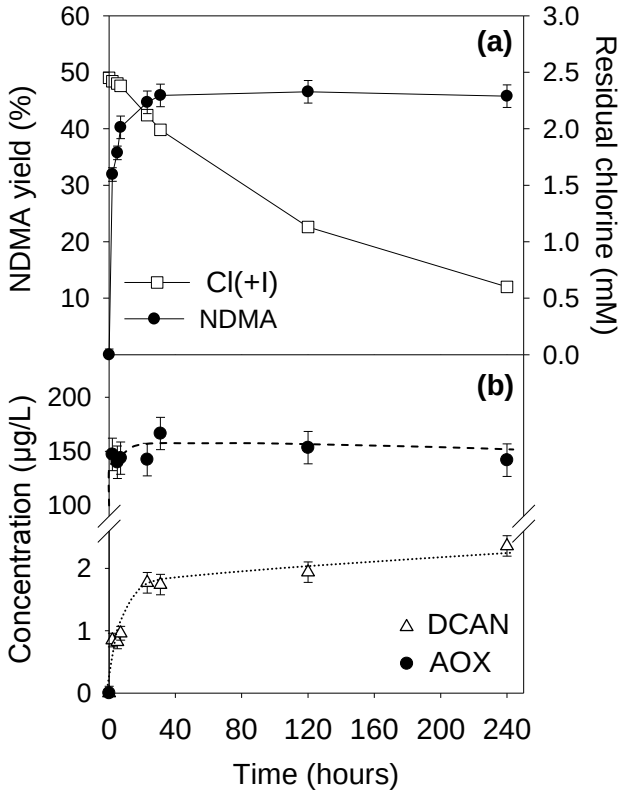
558

559

Table 3. Effect of pH on NDMA and chlorinated DBPs formation from 3 μM ranitidine and 2.5 mM NH_2Cl (5 days contact time).

pH	Molar yield (%)						AOX formation rate (mol/mol)
	NDMA	**DCAN**	**TCAN**	**TCNM**	**1,1-DCP**	**1,1,1-TCP**	
4	0.2	1.33	0.31	12.57	N.D.	N.D.	1.63
5.5	20.6	1.08	0.49	6.14	1.06	0.38	1.63
7	42.2	1.65	0.31	3.05	1.51	1.37	1.74
7.9	59.6	0.81	0.28	1.43	0.24	0.03	1.31
8.5	46.6	0.55	0.33	0.70	0.12	N.D.	1.07
10	10.4	0.06	0.61	0.09	N.D.	0.02	0.56

578 **Figures**



579

580 Figure 1. NDMA formation from 3 µM ranitidine at pH 8.5 with 10 mM phosphate buffer, 2.5 mM

581 monochloramine. Error bars represent one standard deviation (n = 3). NDMA molar yields were

582 calculated based upon the initial ranitidine concentration.

583

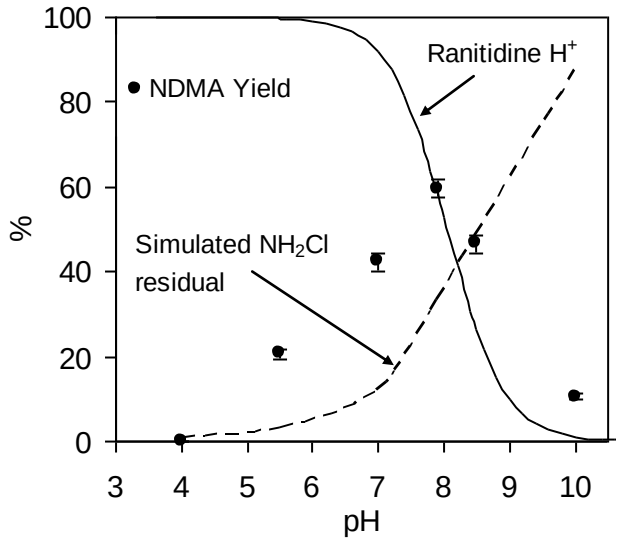


Figure 2. Effect of pH on NDMA formation from 3 μM ranitidine and 2.5 mM monochloramine over 5 days with 10 mM buffer (acetate for pH 4.0-5.5, phosphate for pH 7.0-8.5 and carbonate for pH 10); and NH_2Cl residuals calculated using Jafvert & Valentine model (1992). NDMA yields were calculated based on the initial ranitidine concentration; percentages of NH_2Cl residuals were calculated based on the initial NH_2Cl concentration.

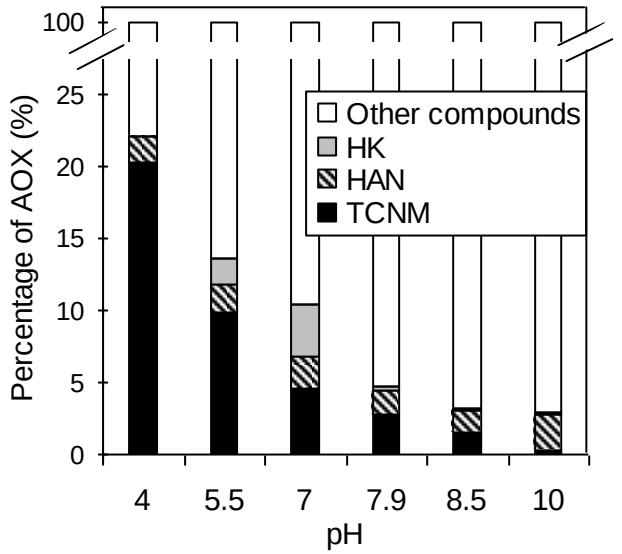
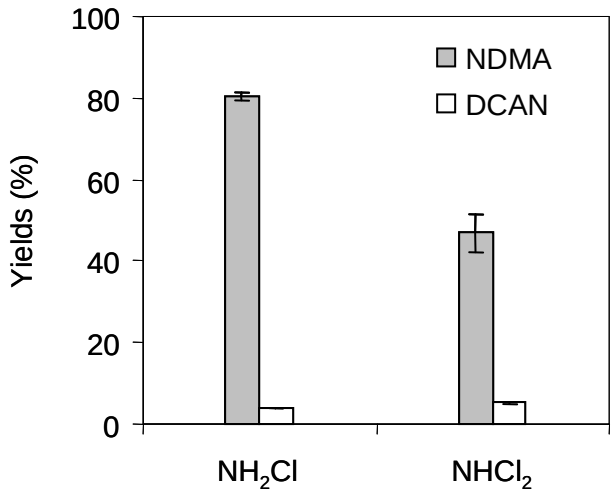


Figure 3. AOX repartition between trichloronitromethane (TCNM), haloacetonitriles (HAN: sum of DCAN and TCAN) and haloketones (HK: sum of 1,1-DCP and 1,1,1-TCP) at different pH from 3 µM ranitidine and 2.5 mM NH_2Cl. Note the scale break.



603

604 Figure 4. NDMA and DCAN formation after 24 h following the application of 1 mM monochloramine

605 or dichloramine to 100 nM ranitidine buffered at pH 8.

606

607

608

609

610

611

612

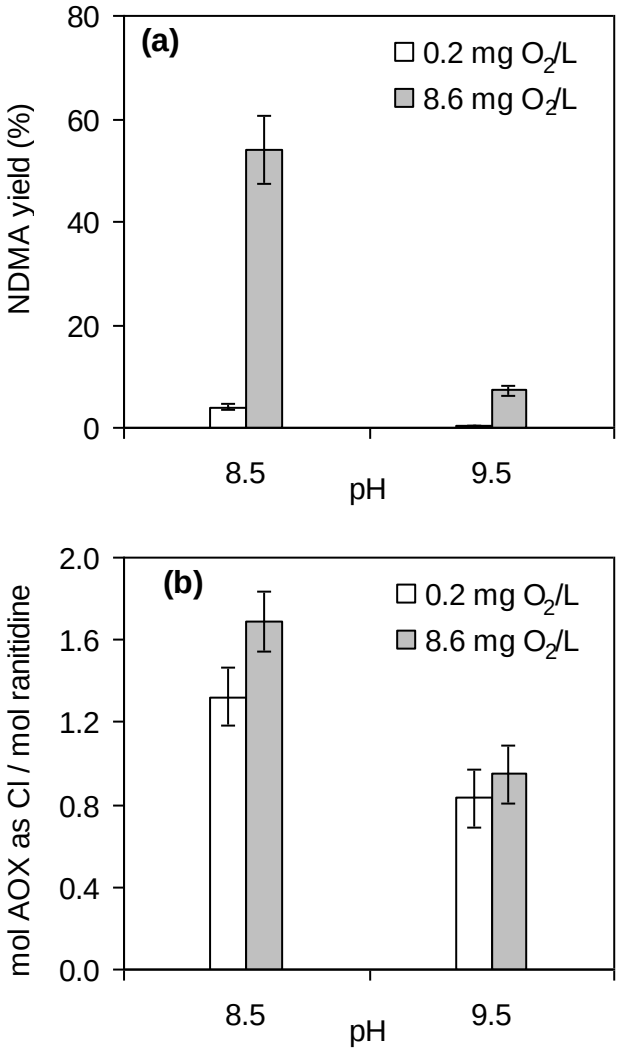
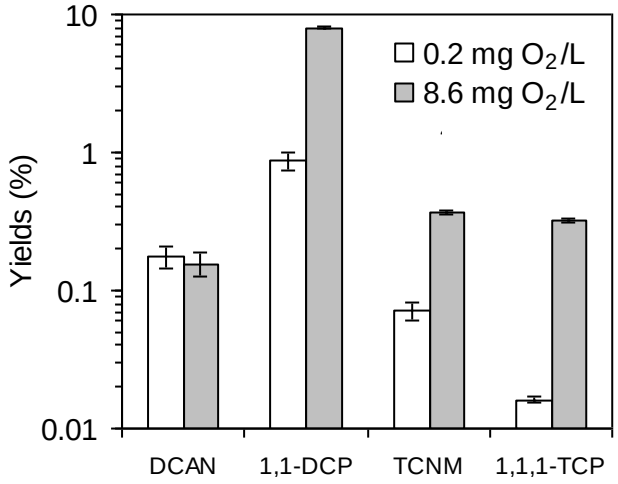


Figure 5. Effect of dissolved oxygen and pH on (a) NDMA and (b) AOX formation from 3 µM ranitidine and 2.7 mM NH_2Cl, over 2 h with 10 mM buffer. NDMA molar yields were calculated based upon the initial ranitidine concentration.



618

619 Figure 6. Effect of dissolved oxygen on DCAN, 1,1-DCP, TCNM and 1,1,1-TCP formation from 3 μM

620 ranitidine and 2.7 mM NH_2Cl over 2 h at pH 8.5 with 10 mM phosphate buffer.